Hoechst Aktiengesellschaft
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Toxikologie

Study of the mutagenic potential of the compound

Octopirox OP. A 038

in strains of Salmonella typhimurium (Ames Test)

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1. Summary

Octopirox OP. A 038 was tested for mutagenicity with the strains TA 100, TA 1538 and TA 98 of Salmonella typhimurium in the presence of exogenous metabolic activation derived from rat liver homogenate. A dose range of 6 different doses from 5 µg/plate to 250 µg/plate was used.

Control plates without mutagen showed that the number of spontaneous revertant colonies was similiar to that described in the literature. All the positive control compounds gave the expected increase in the number of revertant colonies.

Toxicity: The test compound proved to be very toxic to the bacteria at 50 or 100 µg/plate. 250 µg/plate was chosen as top dose level for the mutagenicity study. These are conditions where the test compound can only be tested with very limited sensitivity.

Mutagenicity: In the presence of a metabolic activation system, treatment of the cells with Octopirox OP. A 038 did not result in relevant increases in the number of revertant colonies.

Summarizing, it can be stated that Octopirox OP. A 038 is not mutagenic but cytotoxic in these bacterial test systems using exogenous metabolic activation.

2. Introduction

This report describes experiments performed in a short term test using the procedure of the Salmonella / mammalian-microsome-mutagenicity test (Ames Test) (1,2) to assess the mutagenic potential of the test material in amino acid-dependent strains of Salmonella typhimurium .By the use of liver homogenate the test takes into account the mammalian metabolism of the compound to be tested. The requirement for metabolic activation was investigated by incorporating into the test an activation system by nicotinamide-adenine dinucleotide phosphate ($NADP^+$) -cytochrome P_{450} dependent mixed function oxidase enzymes of the liver. The 9 000 g supernatant of rat liver homogenate has been shown to be very useful in metabolic activation of foreign compounds. The animals were pretreated with Aroclor 1254 as an inducer of several drug metabolizing enzymes (3).

In the Ames test with Salmonella typhimurium strains the effect of the test compound upon the number of back mutations to histidine prototrophy using histidine auxotrophic mutants is investigated. The strain TA 100 was originally derived by a substitution mutation, the strains TA 1538 and TA 98 by frame shift mutations from histidine prototrophic bacteria. All three Salmonella strains are deficient in the complete stucture of their lipopolysaccharide layer and in DNA excision repair system (2). TA 98 and TA 100 possess a modified post-replication DNA repair system which frequently causes an increase in the rate of mutations (4).

3. Material and Methods

Octopirox Charge W 020 has been received as a white powder. It was stored in the dark at room temperature. At the day of the experiment the test substance was dissolved in DMSO at appropriate concentrations.

Preparation and storage of liver homogenate fraction ("S-9")

Male Sprague Dawley rats (200 - 300 g) receive a single intraperitoneal injection of Aroclor 1254 (500 mg/kg body weight) 5 days before sacrifice. Preparation is performed at 0 to 4°C using cold sterile solutions and glassware. The livers from at least 5 - 6 animals are removed and pooled, washed in 150 mM KCl (approximately 1 ml/g wet livers). The washed livers are cut into small pieces and homogenised in three volumes of KCl. The homogenate is centrifuged at 9 000 g for 10 minutes. The supernatant is the S-9 fraction. It is divided into small portions, rapidly frozen and stored at -80°C for not longer than 3 months.

Preparation of S-9 Mix and concentration of cofactors

Sufficient S-9 fraction is thawed immediately before each test at room temperature. One volume of the S-9 fraction are mixed with 9 volumes of the S-9 cofactor solution and kept on ice until used. This preparation is termed S-9 Mix. The concentrations of the different compounds in the S-9 Mix are:

8 mM MgCl
33 mM KCl
5 mM glucose-6-phosphate
4 mM $NADP^+$
100 mM phosphate buffer pH 7,4

Bacteria

Bacteria are grown overnight in nutrient broth (25 g Oxid Nutrient Broth No 2/ liter) at 37°C. The suitable amount of bacteria in the cell suspension is checked by nephelometry. For inoculation, stock cultures which are stored at - 80°C, are used. The compound is tested with the strains Salmonella typhimurium TA 98, TA 100 and TA 1538. Identification of the different bacterial strains is performed periodically as described (2,3).

Mutagenicity experiments

Top agar is prepared for the Salmonella strains by mixing 100 ml agar (0,6 % agar, 0,5 % NaCl) with 5 ml of a 1,0 mM histidine and 5 ml of 1,0 mM biotin solution. The following ingredients are added (in order) to 2 ml of molten top agar at 45°C:

0,1 ml test compound solution
0,1 ml of an overnight nutrient broth culture of the bacterial tester strain
0,5 ml S-9 Mix

After mixing, the liquid is poured into a petridish with minimal agar (1,5 % agar, Vogel-Bonner E medium with 2 % glucose). After incubation for 48 to 72 hours at 37°C in the dark, colonies (his^+ revertants) are counted.

Positive controls

Positive control plates were included for each strain. The following substances were used as positive controls.

Benzo[a]pyrene: TA 98, TA 100, TA 1538
2-Aminoanthracene: TA 98, TA 100, TA 1538

Toxicity experiments and dose range finding

A reduced rate of spontaneously occurring colonies as well as visible thinning of the bacterial lawn were used as indicator for toxicity. Thinning of the bacterial lawn was controlled microscopically.

In combination with the mutagenicity test, toxicity testing was performed as follows: 0,1 ml of the different dilutions of the test compound were thoroughly mixed with 0,1 ml of 10^{-5} dilution of the overnight culture of TA 100 and plated with histidine and biotin rich top agar (3 plates per dose). The solvent control is compared with the number of colonies per plate in the presence of the test compound. Results are given as a ratio of these values. (= surviving fraction).

4. Results

Octopirox OP. A 038 was tested for mutagenicity with Salmonella typhimurium strains TA 100, TA 1538 and TA 98 with the addition of a metabolic activation system. The results obtained with the test material and positive control compounds are presented in table 1 to 4. The number of colonies per plate with each strain as well as mean values of 3 plates, corrected to the next whole number are given.

I. Sterility checks and control plates

Sterility of S-9 mix and the test compound was indicated by the absence of contamination on test material and S-9 mix sterility check plates. Control plates (background control and positive controls) gave the expected number of colonies.

II. Toxicity test:

The test compound was tested at doses of 5 to 250 µg/plate and proved to be very toxic to the bacteria at 50 µg/plate or 100 µg/plate. Thinning of the bacterial lawn and a reduction in the number of colonies have been observed at 50 or 100 µg/plate. According to the data of the surviving fraction toxicity occured with TA 100 even at doses below 50 µg/plate. These are conditions where the test compound can only be tested with very limited sensitivity.

For mutagenicity testing 250 µg/plate was chosen as the highest dose.

III. Mutagenicity test with Octopirox OP. A 038

The test compound did not cause a significant increase in the number of revertant colonies with any of the tester strains in the presence of S-9 mix. No dose dependent effect was obtained (Table 1 - 3).

It is concluded that the test substance is not mutagenic in these bacterial test systems in the presence of an exogenous metabolizing system.

This test was performed according to the methods described. No unforeseen circumstances were observed which have affected the quality and integrity of this study.

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Table 1: Mutagenicity experiment with Octopirox OP. A 038 with metabolic activation

TA 100

Number of revertant colonies per plate and mean values using Salmonella typhimurium strain TA 100

Dose (µg/plate)	Metabolic activation	Mean value	Colonies per plate	Surviving fraction
0 (DMSO)	+	135	156, 125, 125, 135	1.0
5	+	127	118, 120, 130, 134	0.4
12,5	+	121	121, 126, 121, 115	0.4
25	+	89	110, 77, 83, 85	0.1
50	+	50	49, 55, 50, 46, *	0.04
100	+	22	24, 13, 23, 29, **	0.01
250	+		**, **, **, **	0.0

\+ : presence

* : incomplete bacterial lawn

** : no bacterial lawn

Table 2: Mutagenicity experiment with Octopirox OP. A 038 with metabolic activation

TA 1538

Number of revertant colonies per plate and mean values using Salmonella typhimurium strain TA 1538

Dose (µg/plate)	Metabolic activation	Mean value	Colonies per plate
0 (DMSO)	+	13	16, 17, 11, 7
5	+	17	19, 14, 16, 18
12,5	+	14	12, 17, 16, 12
25	+	16	11, 15, 22, 15
50	+	16	13, 19, 15, 15, *
100	+	8	8, 6, 9, 8, **
250	+		**, **, **, **

\+ : presence

* : incomplete baterial lawn

** : no bacterial lawn

Table 3: Mutagenicity experiment with Octopirox OP. A 038 with metabolic activation

TA 98

Number of revertant colonies per plate and mean values using Salmonella typhimurium strain TA 98

Dose (µg/plate)	Metabolic activation	Mean value	Colonies per plate
0 (DMSO)	+	31	42, 34, 25, 24
5	+	28	28, 24, 30, 30
12,5	+	31	33, 34, 26, 31
25	+	30	29, 36, 31, 35
50	+	28	28, 31, 20, 23
100	+	22	16, 30, 29, 21, *
250	+	4	3, 4, 5, 2, *

\+ : presence

* : incomplete bacterial lawn

** : no bacterial lawn

Table 4: mutability (positive controls) and sterility test of the experiment with Octopirox OP. A 038

Number of revertant colonies obtained and mean values using Salmonella typhimurium

Strain	Compound	Dose (µg/plate)	Metab. activition	Mean values	Colonies per plate
TA 100	Aminoanthra-cene	1	+	1330	1570, 1780, 991, 998
TA 1538	Aminoanthra-cene	1	+	881	819, 837, 920, 949
TA 98	Aminoanthra-cene	1	+	652	685, 615, 620, 689
TA 100	Benzo[a]pyrene	10	+	1408	1980, 1780, 882, 989
TA 1538	Benzo[a]pyrene	10	+	252	229, 250, 291, 239
TA 98	Benzo[a]pyrene	10	+	797	833, 820, 787, 750
-	S-9 mix	500 µl	+	0	0, 0, 0
-	Octopirox OP. A 038	250 µg	+	0	0, 0, 0

5. References

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